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Differential Effects of Nephrotoxic Agents on Renal Transport and Metabolism by Use of *In Vitro* Techniques

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A number of studies by the author and other investigators are reviewed in which the *in vitro* kidney slice technique has been used to evaluate the nephrotoxicity of various compounds. The kidney slice technique can be used to determine the effect of prior drug treatment of laboratory animals on renal organic acid (*p*-aminohippurate) or organic base (*N*-methylnicotinamide) transport, on glucose synthesis, and on oxygen consumption by renal coritical slices. The nephrotoxic agents uranyl nitrate and potassium dichromate exert inhibitory effects on renal function, although both agents enhance organic base transport at low doses and potassium dichromate enhances organic acid transport at moderate doses. Enhanced PAH transport has been found to be a sensitive indicator of gentamicin induced nephrotoxicity, while inhibition of other parameters has been reported. The tissue slice method is less effective in evaluating chronic nephrotoxicity such as that produced by lead. The inhibitory effect of mercurial diuretics has been shown to be due to the general depression of metabolic activity by mercury. The kidney slice technique has been found to be a sensitive indicator in the assessment of halogenated hydrocarbon-induced nephrotoxicity. Differential effects of compounds on *in vitro* organic acid and base trasport provides information about the transport of these compounds as well as about their nephrotoxicity. Although it is often desirable to perform *in vivo* tests or other *in vitro* renal function tests, the kidney slice technique has proved to be extremely useful in toxicological evaluations.

Introduction

The kidney is of paramount importance in the excretion of many drugs and chemicals. Investigation of the possible renal toxicity of such agents aids in identifying substances hazardous to health, and also provides information about the mechanisms involved in renal function. Our work has had two primary objectives; first, to study the potential nephrotoxicity of various drugs or other substances in normal experimental animals, and in some cases, in animals in which some degree of renal failure has been experimentally produced; and secondly, to study the properties and mechanisms involved in the renal transport systems for organic acids and organic bases.

One of the methods used to study the secretory mechanisms for organic acids and bases is based on an *in vitro* technique developed by Cross and Taggart (*1*) utilizing renal cortical slices. *p*-Aminohippurate (PAH) is normally used as the prototype for organic acids, while tetraethylammonium (TEA) and *N*-methylnicotinamide (NMN) are the two most commonly used organic bases. Renal cortical slices are prepared from the excised kidneys after drug treatment of the animals for varying lengths of time. The tissue slices are incubated for 90 min under 100% O_2 in an isotonic medium containing PAH and ^{14}O-NMN or ^{14}C-TEA. After incubation, the PAH content of

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the slices and medium is analyzed colorimetrically, while the organic base slice and medium content is determined using a scintillation counter, and the active uptake of each of these compounds by kidney slices is reported asthe slice to medium, or S/M ratio.

A simplified illustration of our general approach in such studies is shown in Figure 1. In addition to measurement of PAH-NMN accumulation, a number of other measurements of renal function have also been utilized in our laboratory. Glucose synthesis by renal cortical slices was determined after incubation under 95% O_2–5% CO_2 for 60 min, with results expressed as umole glucose/g tissue wet weight/hr. Oxygen consumption by tissue slices was measured with a Warburg apparatus by use of conventional manometric techniques (*1*). Urines may be collected during drug treatment for measurement of volume, osmolality or urinary enzymes, and blood samples obtained for determination of BUN, serum creatinine or serum enzyme levels.

The kidney slice technique has been used for many *in vitro* studies of renal function and metabolism since this system offers several advantages: most of the cells are intact; preparation of experimental samples is straightforward and samples are easy to handle; the incubation medium can be manipulated to study effects such as substrate or ion changes; by pooling slices from several animals or alternatively dividing slices from a given animal for use in several flasks one can compare various parameters directly while minimizing tissue variability; and physiological factors such as tubular obstruction, dehydration, or blood pressure changes are eliminated.

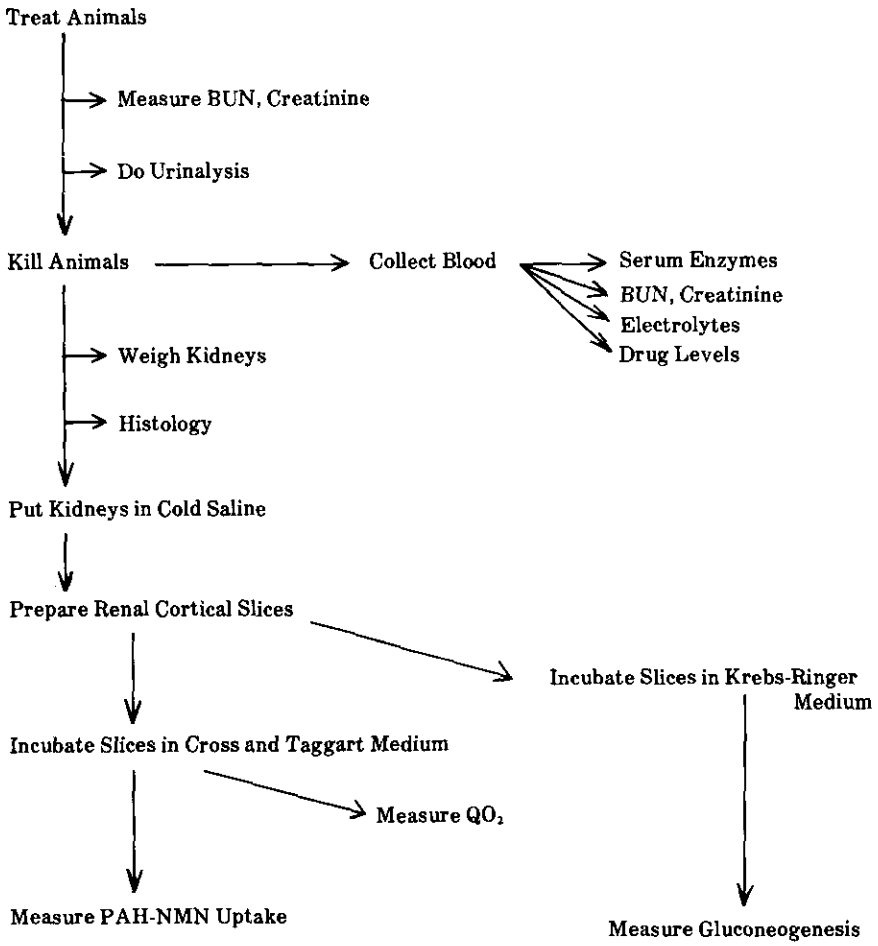

FIGURE 1. *Protocol for renal function tests.*

Studies with Uranyl Nitrate and Potassium Dichromate

In the course of performing renal function tests following the adminstration of various nephrotoxic agents, we observed that treatment of adult male rats with uranyl nitrate specifically increased NMN uptake by rat kidney slices, but had no effect on PAH accumulation (Fig. 2). Rats were killed 48 hr after a single IP injection of 6 mg/kg uranyl nitrate. Body weight was the same in control and treated rats, indicating that the increased kidney weight: body weight ratio represented increased kidney weight. Additional experiments demonstrated a general effect of uranyl nitrate on organic base transport, since both TEA and NMN accumulation were enhanced in cortical slices 24 hr after uranyl nitrate treatment (2).

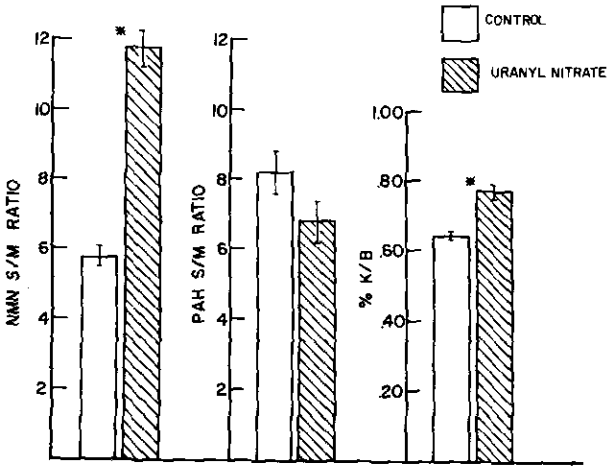

FIGURE 2. Effect of uranyl nitrate treatment of adult male rats on uptake of NMN and PAH by renal cortical slices, and on the kidney weight to body weight ratio. Each bar represents the mean ± S.E. obtained with duplicate determinations from nine animals. The asterisks indicate values significantly different from their respective controls ($p<0.05$). From Hirsch (2) with permission of the Canadian Journal of Physiology and Pharmacology.

Uranyl nitrate is nephrotoxic at 6 mg/kg, and the toxicity increases as the time after injection is prolonged. The data shown in Figure 3 demonstrate that doses of uranyl nitrate as low as 0.5 and 1.0 mg/kg were able specifically to stimulate NMN uptake by renal cortical slices when measured 24 and 48 hr after injection. The degree of nephrotoxicity elicited by uranyl nitrate was not

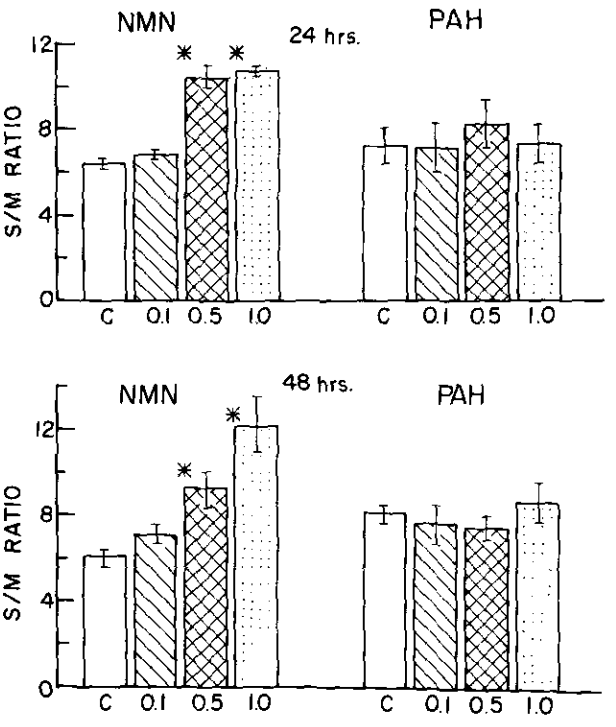

FIGURE 3. Effect of uranyl nitrate treatment of adult male rats on uptake of NMN and PAH by renal cortical slices. Rats were killed 24 or 48 hr after a single IP injection of saline (c) or 0.1 0.5, or 1.0 mg/kg uranyl nitrate. Each bar represents the mean ± S.E. obtained with duplicate determinations from five animals. The asterisks indicate values significantly different from their respective controls ($p<0.05$). From Hirsch (2) with permission of the Canadian Journal of Physiology and Pharmacology.

directly related to the degree of stimulation of NMN transport, however. At 0.5 mg/kg, some cellular swelling of the proximal tubular cells could be seen, with isolated areas of cellular necrosis (Fig. 4*b*). When 6 mg/kg uranyl nitrate was administered, extensive cellular necrosis was evident (Fig. 4*c*) although the degree of NMN stimulation produced at these two doses was comparable.

It was then of interest to evaluate in greater detail the effects produced by another nephrotoxin, potassium dichromate. Although the effect was similar in general to the uranyl nitrate response, dichromate produced some interesting differences. At 24 hr after a single subcutaneous injection of a low dose of potassium dichromate (5 mg/kg), the PAH S/M ratio was significantly increased, while NMN uptake was enhanced in renal cortical slices after treatment of rats with 25 or 40 mg/kg potassium dichromate (Fig. 5).

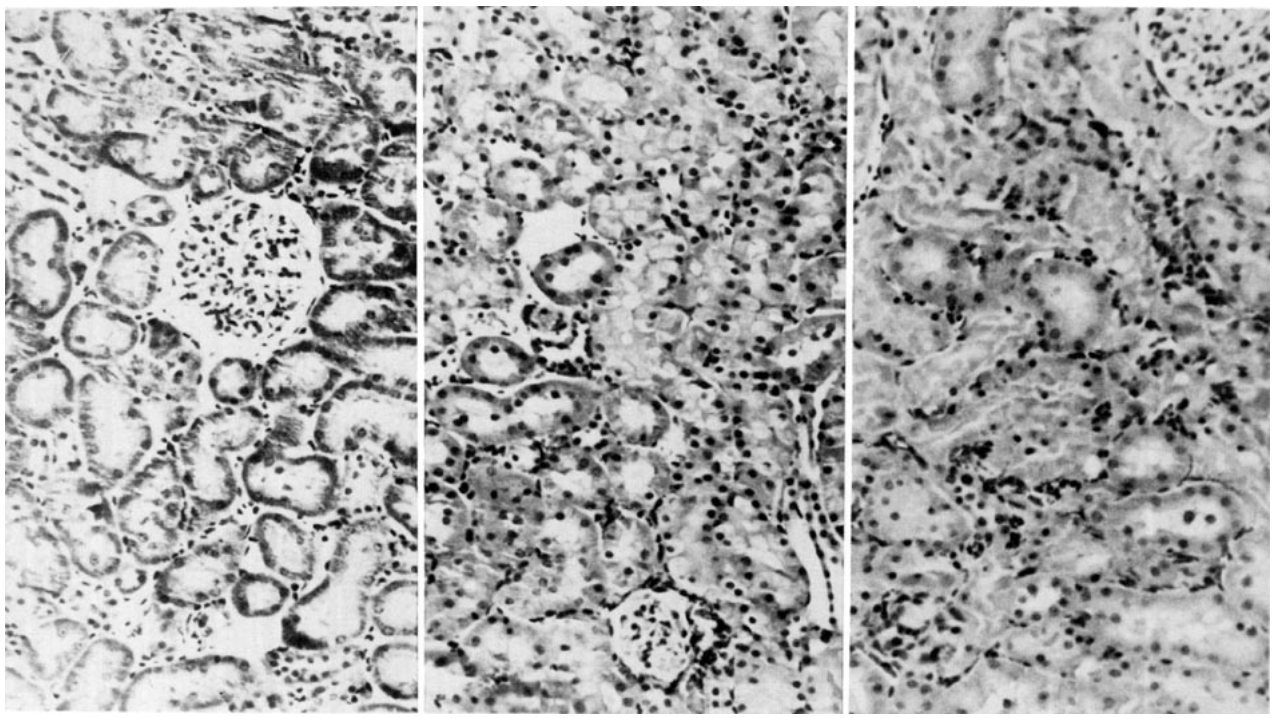

FIGURE 4. *Effect of uranyl nitrate treatment on kidneys from adult male rats: (a) control, (b) 48 hr after 0.5 mg/kg uranyl nitrate, (c)* 48 hr after 6 mg/kg uranyl nitrate. Magnification ×80. From Hirsch (*2*) with permission of the Canadian Journal of Physiology and Pharmacology.

Significant increases in the kidney weight: body weight ratio and tissue water content were observed. The nephrotoxicity of potassium dichromate was shown by the increased tissue water content and by the significant depression of the PAH S/M ratio at 25 and 40 mg/kg.

Only depression of ion uptake was observed 48 hr after potassium dichromate injection (Fig. 6). The cellular toxicity produced by dichromate at this time was extensive and severe. No stimulation of NMN accumulation was produced, and at 40 mg/kg both PAH and NMN uptake were significantly depressed. It appears that there is a fine distinction between the nephrotoxic property of dichromate and its ability to induce organic base transport. At 48 hr after injection, renal necrosis apparently is severe enough to interfere with the stimulatory action of dichromate on base transport. Thus, although both uranyl nitrate and potassium dichromate produce renal damage, it appears that this effect is not entirely the same as the effect of these compounds on organic base transport.

The action of dichromate on renal metabolism was also investigated by adding potassium dichromate *in vitro* to incubation beakers (*3*). PAH uptake by renal cortical slices was increased at a

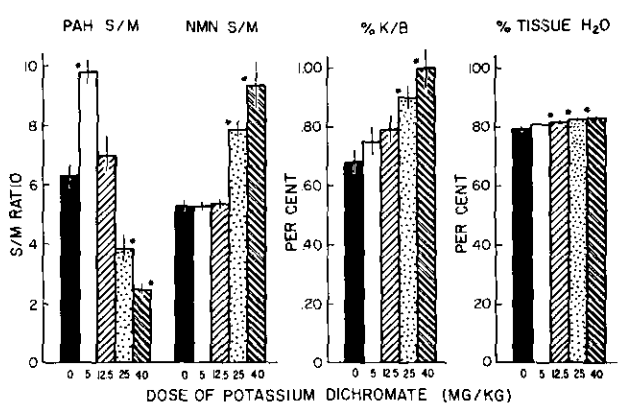

FIGURE 5. PAH and NMN accumulation by rat renal cortical slices, rat kidney weight to body weight ratio and kidney water content 24 hr after a single *SC* injection of adult male rats with potassium dichromate. Each bar represents the mean ± S.E. of determinations from six to eight animals. The asterisks indicate values significantly different from their respective controls ($p<0.05$). From Hirsch (*3*) with permission of the *Journal of Pharmacology and Experimental* Therapeutics.

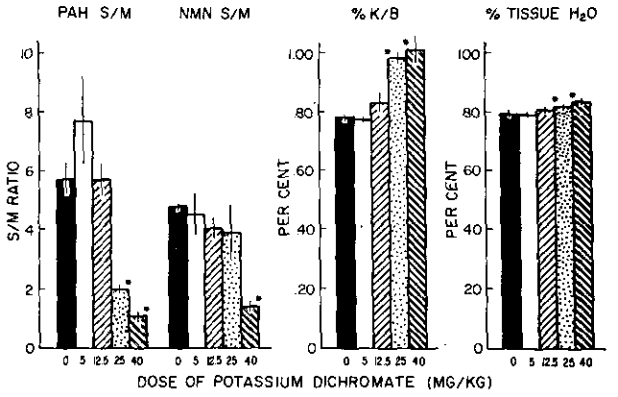

FIGURE 6. PAH and NMN accumulation by renal cortical slices, rat kidney weight to body weight ratio and kidney water content 48 hr after a single SC injection of adult male rats with potassium dichromate. Each bar represents the mean ± S.E. of determinations from three rats. The asterisks indicate values significantly different from their respective controls ($p<0.05$). From Hirsch (*3*) with permission of the Journal of Pharmacology and Experimental Therapeutics.

concentration of $10^{-6}M$ potassium dichromate and was decreased at $10^{-3}M$ dichromate. QO_2 values showed a progressive decline as dichromate concentration increased. In contrast, the NMN S/M ratio increased as the concentration of dichromate increased, until at $10^{-4}M$ the value was significantly greater than control (*3*). At $10^{-3}M$ potassium dichromate, all of the measured parameters including NMN uptake, were inhibited to a significant degree. Thus these *in vitro* observations with PAH and NMN parallel the results obtained after treating the rats with dichromate. At concentrations greater than $10^{-4}M$ or at doses greater than 25 mg/kg at 48 hr, the nephrotoxic effect of dichromate overwhelms its apparent stimulatory action on base transport.

The effects of potassium dichromate on a number of other parameters of renal metabolism have also been determined. BUN levels were significantly increased, while glucose synthesis by renal cortical slices was inhibited when measured 24 hr after injection of 25 or 40 mg/kg potassium dichromate (*3*).

In some experiments, the runout or efflux of ^{14}C-NMN or ^{14}C-PAH was studied in slices after preloading them with NMN or PAH for 90 min (*4*). Slices were then transferred through a series of 12 beakers containing no NMN or PAH and the data expressed as DPM of NMN or PAH remaining in the slices as a function of the runout beaker number (time). The first-order NMN runout constant for slices from treated animals was significantly less than that for control slices (0.022 vs. 0.045, $p<0.05$). The initial concentration of NMN was higher in slices from treated rats, and the rate of runout was significantly less (*4*). PAH runout was similar in slices from control rats and rats injected with 5 mg/kg dichromate, but was significantly greater than control in slices from rats given 40 mg/kg dichromate (*4*). This is consistent with the observation that 40 mg/kg potassium dichromate produced substantial inhibition of renal slice PAH content (i.e., the PAH S/M ratio).

Intravenous injection of rats with NMN results in higher NMN cortex/serum (C/S) ratios in rats that had been given 40 mg/kg potassium dichromate 24 hr earlier (Fig. 7). Rats were killed 30 min after the intravenous injection of C^{14}-NMN, aliquots of serum and kidney cortex were digested in soluene (Packard Instrument Company), and radioactivity then determined. In addition to the higher C/S ratios in the rats, urinary NMN

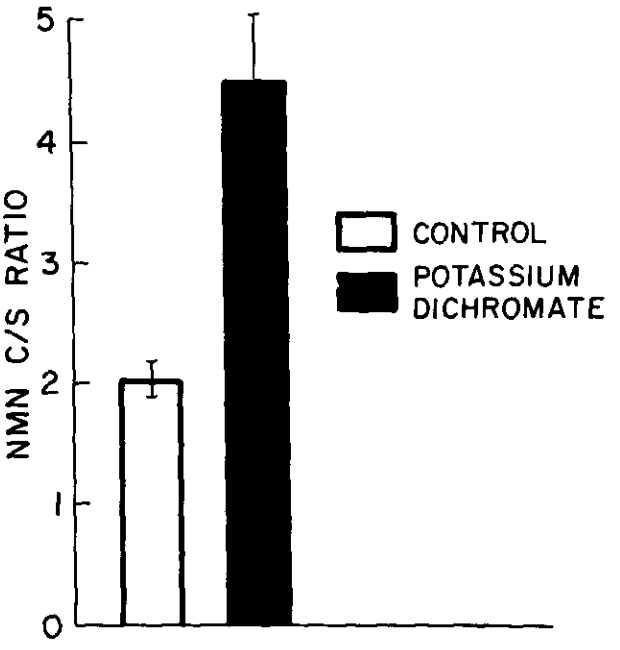

FIGURE 7. *In vivo* accumulation of ^{14}C-NMN (13 μCi/kg, IV) in kidneys of rats treated 24 hr earlier with saline or 40 mg/kg potassium dichromate. Rats were killed 30 min following the NMN injection, the concentration of ^{14}C-NMN in the renal cortex and serum then determined and the results expressed as the cortex/serum (C/S) ratio. Each bar represents the mean ± S.E. from six rats. The C/S ratio of the treated animals is significantly greater than that from control animals ($p<0.05$). From Hirsch and Pakuts (*5*) with permission of Toxicology and Applied Pharmacology.

excretion was substantially less than in control animals. Stimulation of the NMN C/S ratio *in vivo*, and the decreased runout of NMN *in vitro*, indicate that the enhanced NMN accumulation occurring after potassium dichromate is related to enhanced organic base retention in the renal cortical cells. Whether this retention is due to specific binding of NMN by organic base receptors, or merely binding of NMN to denatured protein, has not been established. Nevertheless, it is evident that the *in vivo* experiment corroborates in the *in vitro* observations with kidney slices.

Although both uranyl nitrate and potassium dichromate enhanced NMN accumulation by renal cortical slices, stimulation of this proximal tubular transport system does not appear to be simply a nonspecific response to cellular injury. Administration of mercuric chloride (2.5 or 5 mg/kg) produced renal damage that was both time and dose related as measured by histological and metabolic techniques (*3*). PAH and NMN S/M ratios were not enhanced when mercuric chloride-induced nephrotoxicity was produced, suggesting that this nephrotoxin may act at different sites in the renal tubular cells than uranyl nitrate or potassium dichromate. These observations also indicate that the changes in *in vitro* uptake of PAH and NMN produced by other nephrotoxins such as uranyl nitrate and potassium dichromate can serve as sensitive indicators of nephrotoxicity.

Studies with Gentamicin

The *in vitro* slice technique has also been utilized to evaluate the nephrotoxicity of gentamicin, since there have been a number of clinical reports of gentamicin toxicity (*6,7*). Gentamicin is an aminoglycoside antibiotic related to neomycin, kanamycin, and streptomycin. Like these other antibiotics, gentamicin is ototoxic, and vestibular damage has been reported, particularly in patients with impaired renal function (*8*). Next to vestibular damage, nephrotoxicity is the most important side effect of gentamicin. After parenteral administration, elimination of gentamicin is primarily via the kidneys by glomerular filtration. Some 80-90% of an injected dose is recovered in the active, unchanged form in the urine, leading to high urinary concentrations (*9*). In patients with renal failure, prolonged elevation of plasma gentamicin levels is observed due to its decreased clearance (*10*). The early signs of nephrotoxicity in patients are elevated BUN and plasma creatinine levels.

In using the *in vitro* slice technique to investigate the possible nephrotoxicity of gentamicin, Hirsch (*11*) made the following observations. At the lowest dose used (15 mg/kg) no significant changes were observed in the parameters measured after 3 weeks of treatment. At 40 mg/kg some indications of renal toxicity were evident: glucose synthesis and O_2 consumption were inhibited, and the kidney weight:body weight ratio was increased. NMN transport, glucose synthesis, and changes in kidney weight appeared to be most sensitive to gentamicin treatment; these parameters were also significantly different from control after 1 or 2 weeks of treatment at 70 mg/kg gentamicin. PAH accumulation and BUN levels were not changed (*11*).

Exacerbation of gentamicin nephrotoxicity by methoxyflurane has been reported by Barr et al. (*12*), and Hirsch (*11*) has also shown that gentamicin toxicity was increased in rats suffering from glycerol-induced renal failure. Gentamicin alone (70 mg/kg IM for 8 days) produced changes only in kidney weight and glucose synthesis while glycerol (1 ml/100 g of 50% glycerol in saline sc on days 1 and 8) produced no changes. Administration of both compounds, however, caused increased BUN levels and kidney weight, reduced PAH-NMN transport by kidney slices, and inhibited glucose synthesis. Measurement of gentamicin in serum confirmed the *in vitro* slice observations in that the gentamicin serum concentrations were significantly higher when both gentamicin and glycerol were administered (*11*). Thus, the *in vitro* method accurately demonstrated the changes in toxicity and reflected the changes in gentamicin serum concentration.

Recently Cohen et al. (*13*) completed a gentamicin study on Sprague-Dawley rats. They examined the effects of gentamicin on several parameters of renal function in an attempt to identify "the early functional correlates of gentamicin toxicity." BUN and serum creatinine levels were somewhat elevated after 4 days of gentamicin treatment at 100 mg/kg, while the urinary concentrating capacity was reduced. Gentamicin nephrotoxicity has been reported (*14*) to be associated with structural changes in the proximal tubules. Cohen et al. (*13*) found that gentamicin-treated rats secreted PAH at a higher rate than control animals. The results observed in their *in vivo* experiments were then confirmed by *in vitro* studies in which renal cortical slices of rats treated with gentamicin (100 mg/kg/day, *im*) accumulated PAH to a greater degree than

the slices from control animals. The PAH S/M ratio was enhanced to the greatest extent after 4 days of treatment, with incubation time varying from 60 to 180 min. No discernable effect on organic base transport was observed (*13*).

The production of nephrotoxicity implies the development of impaired renal function, usually accompanied by histologic changes. The relationship of enhanced PAH transport to the subsequent appearance of renal failure and proximal tubular necrosis is not clear. The same statement can be made in reference to the enhancement of NMN accumulation by uranyl nitrate or potassium dichromate (*2,3*).

The kidney slice technique was also used to illustrate differences in sensitivity to gentamicin in different strains of rats. In an effort to utilize doses closer to that used in humans, we began using the Fisher 344 strain of rats in our gentamicin studies. This rat strain has been reported to be more sensitive to a number of drugs, including gentamicin (*14*). In one series of experiments, Fisher 344 rats were treated with 10 mg/kg IM gentamicin twice daily for 15 days (Table 1, Series 1). BUN levels were not changed, but kidney weight was increased, and NMN uptake was significantly reduced, suggesting that some nephrotoxicity had been produced using this dosage schedule. PAH accumulation by renal cortical slices from gentamicin treated rats was significantly enhanced when compared to controls (Table 2, Series 1), supporting the observations of Cohen et al. (*13*). In another set of experiments, the treatment duration for gentamicin was 4 days (twice daily at 10 mg/kg). The results (Table 1, series 2) suggest that substantial nephrotoxicity was not produced since kidney weight, BUN levels and NMN transport were not significantly different from control values. However, the PAH S/M ratio was still significantly enhanced after gentamicin treatment.

Since the earlier experiments had demonstrated that gentamicin produced only moderate nephrotoxicity when given at 10 mg/kg twice daily for 2 weeks, the same dose was administered three times daily for 2 weeks (Table 2, series 3). Nephrotoxicity was quite evident now, with kidney weight, BUN levels, and serum creatinine levels all being significantly greater than control values. As expected, the NMN S/M ratio was reduced. The PAH response was different from that seen earlier, however, since the PAH S/M ratio now was significantly lower than control. Thus, in this experiment in which gentamicin induced substantial nephrotoxicity, a reduction in organic acid transport was now produced. It can be seen that both NMN and PAH S/M ratios can serve as useful indicators of the degree of nephrotoxicity produced by gentamicin.

Histological observations support the slice studies on PAH and NMN uptake. When gentamicin was given at 10 mg/kg twice daily for 2 weeks, minimal nephrotoxicity was produced. When the gentamicin dose was increased to 10 mg/kg three times daily for 2 weeks, obvious toxicity was produced (Fig. 8). Proximal tubules showed varying degrees of cellular swelling vacuolar degeneration, necrosis and desquamation of epithelial cells. These changes were even more evident in kidneys from rats given 40 mg/kg twice daily for 8 days (Fig. 8).

The above data suggest that the effects of gentamicin on PAH transport can be dissociated from the appearance of renal failure. It is evident that high doses of gentamicin which produce marked nephrotoxicity inhibit PAH transport (and other parameters of renal metabolism also). This is similar to the effect of potassium

Table 1. Effect of gentamicin on various parameters of renal function in Fischer 344 rats.

	Series 1, 15 days[a]		Series 2, 4 days[b]		Series 3, 14 days[c]	
	Control	Gentamicin	Control	Gentamicin	Control	Gentamicin
Kidney wt, g/100 g	0.71±0.01	0.93±0.03[d]	0.69±0.02	0.73±0.03	0.67±0.02	1.01±0.01[d]
BUN, mg/100 ml	19±3.5	20±1.2	20±0.4	21±1.8	20±1.0	27±2.0[d]
Serum creatinine, mg/100 ml	—	—	—	—	0.32±0.04	0.73±0.06[d]
PAH S/M	7.7±0.3	10.8±0.8[d]	10.3±0.4	14.7±1.7[d]	11.1±0.07	5.8±0.4[d]
NMN S/M	5.7±0.1	3.5±0.6[d]	6.5±0.3	5.2±0.2	6.8±1.0	2.3±0.1[d]

[a] Rats were treated with saline or 10 mg/kg gentamicin SC twice daily for 15 days. Values represent the mean ± S.E. from 4 rats.

[b] Rats were treated with saline or 10 mg/kg gentamicin SC twice daily for 4 days. Values represent the mean ± S.E. from 6 to 8 rats.

[c] Rats were treated with saline or 10 mg/kg gentamicin SC three times daily for 14 days. Values represent the mean ± S.E. from 4 rats.

[d] Values significantly different from their respective controls ($p<0.05$).

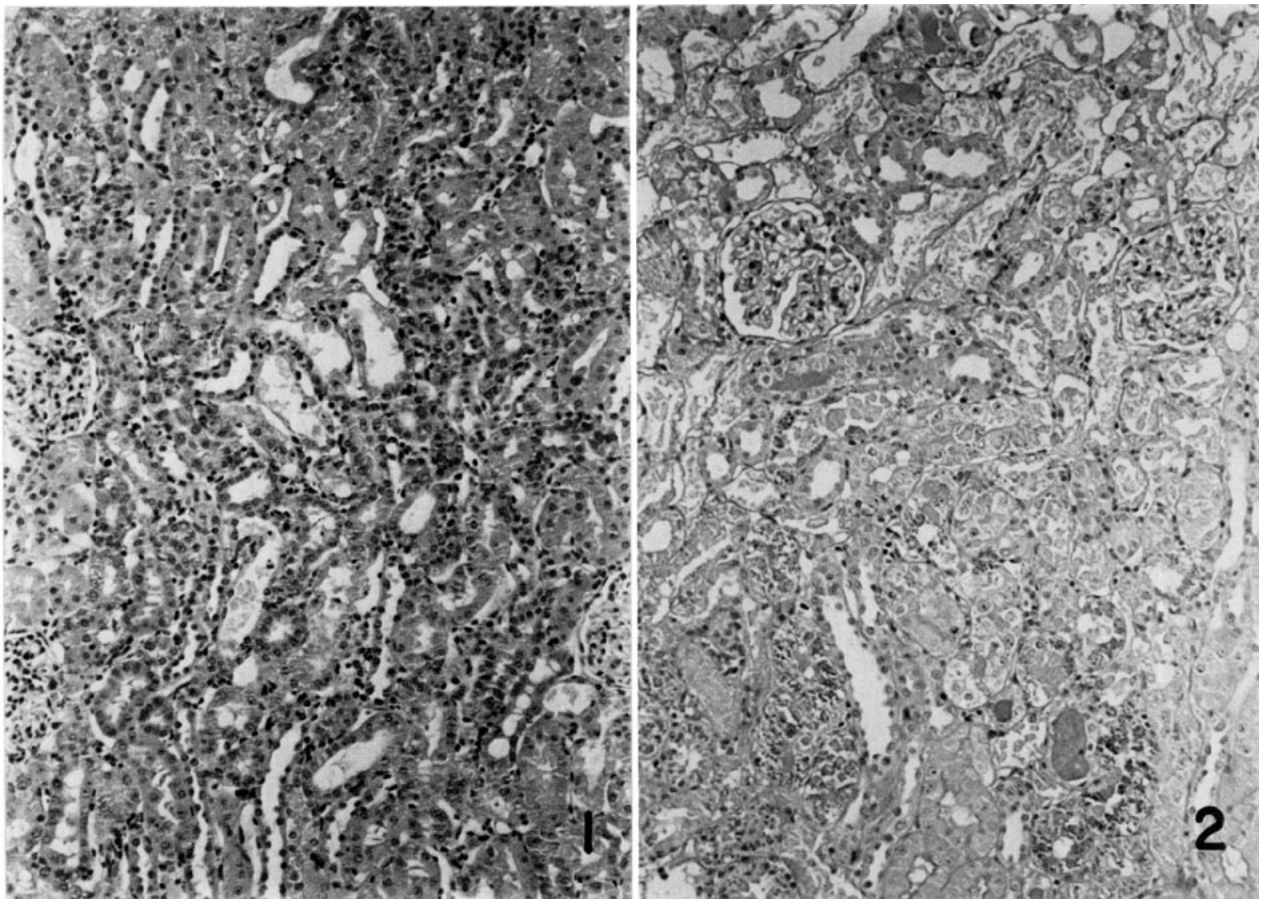

FIGURE 8. Effects of gentamicin on kidneys from Fisher 344 adult male rats: (*1*) 10 mg/kg gentamycin three times daily for 2 weeks; (*2*) 40 mg/kg gentamycin twice daily for 8 days. Magnification ×80.

dichromate, where low doses stimulate PAH while high doses inhibit PAH transport. With gentamicin, low doses which did not produce marked nephrotoxicity still enhanced PAH uptake by renal cortical slices. Thus, the stimulation of the PAH S/M ratio by gentamicin appears to be a sensitive and specific indicator of the renal cellular damage produced by this drug.

Studies with Heavy Metals

While the tissue slice method is quite effective in measuring acute renal tubular necrosis, it is somewhat less effective in assessing chronic toxicity, for example, the toxicity produced by lead. Of course, it must be kept in mind that it is also much more difficult to demonstrate chronic nephrotoxicity using these renal function tests, since the kidneys adapt to accommodate toxicity that has been produced.

In one study, Wistar rats were fed 2% lead acetate in their diets for 10 or 40 weeks (*15*). The kidney weight/body weight ratio was significantly enhanced in treated rats, while gluconeogenesis and pyruvate decarboxylation were inhibited. However, BUN levels were not changed, and neither organic acid transport nor organic base transport was inhibited by lead exposure. In 10 week old rats exposed to 2 or 4% lead acetate in the diet since birth, the kidney weight/body weight ratios were enhanced but this was largely the result of a decrease in body weight (*15*). Accumulation of PAH and TEA by kidney cortical slices was not markedly altered, although gluconeogenesis was inhibited. The BUN changes were not physiologically important.

The histologic changes in the kidneys of rats receiving lead acetate from birth via the diet or via the milk of their dams have also been eval-

uated (*15*). Some vacuolar degeneration was produced in the distal segment of the proximal tubule in kidneys of 30 day old rats maintained on diets containing 2% lead acetate. In addition to vacuolar degeneration, some cellular necrosis occurred when 4% lead acetate was used. The most pronounced effects were observed when rats were maintained on diets containing 2% lead acetate for the first 10 weeks of life; in this group, lead inclusion bodies were observed in addition to cellular necrosis and sloughing of tubular cells. Thus, the histological changes, while minimal, generally correlated with the results obtained using kidney slices. These studies show that, in this type of situation, histological studies and some *in vivo* function test should be carried out to complement *in vitro* observations.

Gieske and Foulkes (*16*) have utilized the *in vitro* slice technique to study the acute effects of cadmium on proximal tubular function in rabbits. After treatment with cadmium (a single IV injection), cortical slices were incubated in the presence of varying PAH concentrations. The active uptake of PAH by cortical slices from treated rabbits was severely depressed (*16*). This marked inhibition of active PAH transport indicated that the depression of PAH clearance and PAH extraction (observed in parallel *in vivo* studies) resulted from the cytotoxic effects of cadmium rather than simply from changes such as renal blood flow differences. Thus, the *in vitro* approach clarified observations made *in vivo*.

Another study in which the tissue slice method was used to establish the effects of a heavy metal on renal metabolism was done by Hook and Hirsch (*17*). In addition to their well-known effects on the renal transport of inorganic ions, the mercurial diuretics also have pronounced effects on other renal functions, including the organic anion secretory system. Some of the mercurial diuretics are organic acids and are capable of being secreted, but there are some exceptions. Chlormerodrin is not an organic acid and yet it inhibits PAH transport, and some mercurial diuretics also inhibit glucose transport. Dog and rat kidney cortical slices were therefore reflects a general metabolic inhibition rather than a specific effect of mercurial compounds on anionic transport. The two acidic mercurials used, meralluride and mercaptomerin, inhibited the accumulation of the organic base NMN to the same extent as that of PAH in both rat and dog slices (*17*). In addition, the nonacidic mercurial chlormerodrin also reduced both PAH and NMN uptake. The inhibitory potency of all 3 diuretics was approximately the same (50% inhibition near $5\times10^{-4}M$).

Since the S/M ratio of PAH and NMN is measured in a steady state, it was considered possible that the effect of the mercurials was on the retention of organic ions rather than directly on the transport process. Ross and Farah (*19*) demonstrated that the rate of PAH accumulation during very early incubation times can be used to measure the actual rate of transport (since at these early time periods the intracellularly accumulated PAH is not of sufficient concentration to alter the next flux). Both the organic acid mercurial mercaptomerin and the nonacidic chlormerodrin decreased the rate of PAH entry into the rat renal cortical slices (*17*). In another *in vitro* slice study, it was shown that inhibition of PAH and NMN uptake was not restricted to diuretic compounds since mercuric chloride and methylmercury both inhibit uptake of PAH and NMN also, with about 50% inhibition at $10^{-4}M$ (*20*). These slice studies thus demonstrated that the inhibitory effect of organic mercurial compounds on PAH transport is nonspecific, reflecting general depression of metabolic activity by these compounds.

Studies with Halogenated Hydrocarbons

Plaa and his associates have conducted a number of studies to determine if halogenated hydrocarbon-induced nephrotoxicity could be demonstrated using the kidney cortical slice technique. Carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), and trichloroethylene (TCE) were found to be nephrotoxic on kidney slices from mice (*21*). In rats, CCl_4 was most consistently nephrotoxic at sublethal doses, as indicated by inhibition of the PAH S/M ratio (*21*). $CHCl_3$ and TCE were less active in terms of maximum effect (or % inhibition), but were more potent in that they exerted their effects at lower doses (*22*). In mice, $CHCl_3$ was the most effective inhibitor of PAH transport. The minimum subcutaneous dose of $CHCl_3$ that was effective in producing nephrotoxicity in mice was 0.025 ml/kg. Watrous and Plaa (*22*) were able to conclude that $CHCl_3$ is at least 50 times as nephrotoxic in the male mouse as in the male rat.

Halogenated hydrocarbons thus produce functional impairment of renal tubules, as indicated by impairment of renal anionic transport. The kidney slice technique appears to be a more sensitive method for determining nephrotoxicity in the mouse since inhibition could be detected at lower doses. In earlier *in vivo* studies (*23*), no

evidence was found to suggest that CCl_4 produced functional impairment of the kidney as indicated by changes in PSP excretion or glucose reabsorption at doses up to 4 ml/kg. Although this earlier work revealed no evidence that CCl_4 impaired tubular function, there was some histological evidence of renal toxicity. Thus, the *in vitro* slice studies described correspond more closely to the histological observations. *In vivo* techniques such as PSP excretion have a distinct advantage, however, in that repeated measurements can be made on individual animals.

Under certain circumstances, Watrous and Plaa (*21*) found that the nephrotoxicity of halogenated hydrocarbons was decreased when mice were pretreated one or more times with the same solvent. This is analagous to earlier findings by Culliford and Hewitt (*24*) who found that the histopathological changes produced in the kidneys of male mice were reduced by pretreatment with CCl_4 or $CHCl_3$.

The results obtained with kidney slices were further evaluated by Watrous and Plaa (*21*) in an attempt to increase understanding of the mechanisms by which CCl_4 and $CHCl_3$ exerted their nephrotoxic effects. While the hepatoxicity of these solvents may be associated with the formation of free radicals that initiate the autocatalytic breakdown of the smooth endoplasmic reticulum, it was concluded that there was little evidence that the nephrotoxicity of these agents results from metabolites rather than the parent compounds. Kidney slices have been reported to be much less active than liver slices in metabolizing $CHCl_3$ or CCl_4 (*25*).

Other Studies

Hook et al. (*26*) utilized the *in vitro* slice technique to study various factors associated with the toxicity of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). 2,4,5-T is more toxic to dogs than to rats, and Piper et al. (*27*) demonstrated that renal excretion of 2,4,5-T was greater in rats than in dogs. Hook et al. (*26*) showed that addition of 2,4,5-T to the incubation medium produced a dose-related depression of PAH accumulation. Inhibition of PAH uptake by 2,4,5-T appeared to be competitive in nature since the estimated maximal velocity of PAH uptake into rat kidney cortical slices was not changed. The apparent K_m was changed by 2,4,5-T. Thus, these studies demonstrated that, as an organic acid, 2,4,5-T was actively secreted by rat tissue, which would explain the shorter biological half-life in this species. Berndt and Koschier (*28*) have also used the *in vitro* slice method to show that the active renal tubular transport of 2,4,5-T and 2,4-D contributes to the low toxicity of these compounds.

Braun and associates have evaluated the renal toxicity of heme proteins by determing the effects of these heme proteins on renal tubular function, using rat kidney slices (*29*). Depression of organic acid and base transport in kidney slices was the earliest and most consistent proximal tubular dysfunction observed. Other parameters measured included oxygen consumption, ammoniagenesis, and gluconeogenesis by renal cortical slices. Among the various physiological functions studied, only hippurate and TEA transport were decreased at 4 hr after subcutaneous glycerol injection. On the other hand, at 24 hr, PAH and TEA transport and O_2 consumption in kidney slices were significantly decreased (*27*).

In another *in vitro* study, Preuss et al. (*30*) investigated the effects of urinary proteins from patients with myeloma on renal tubular function. Urinary proteins from patients with myeloma had a generalized depressive effect on proximal tubular function while urinary proteins from nephrotic patients did not. This was indicated by the depression of ammoniagenesis and gluconeogenesis, as well as the inhibition of hippurate and TEA uptake seen utilizing serum from myeloma patients (*30*). Preuss et al. also studied (*31*) various parameters of renal function, utilizing *in vitro* techniques to investigate the possibility that some clinical and experimental hypertension is secondary to deranged kidney metabolism. They observed that there are significant differences in PAH transport and oxygen consumption in kidney slices from normotensive and hypertensive rats.

Conclusion

In conclusion, we have tried to present a number of studies in which the *in vitro* kidney slice technique has been used to evaluate or measure the nephrotoxicity produced by a number of different compounds. This technique has proved to be extremely useful in assessing renal toxicity. In many cases it is desirable to do concomitant histological studies, measure serum enzyme, creatinine, or BUN levels, or use various *in vivo* physiological procedures as additional sources of data for toxicological evaluation. There are, of course, a number of disadvantages inherent in the slice technique as an *in vitro* approach, although we have stressed the advantages of this

technique. The *in vitro* approach obviously cannot completely replace *in vivo* methods, but it can be used in many cases to assess renal toxicity, or in some situations to verify or clarify results obtained *in vivo*.

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